Attachment 1

TITLE: **Determination of the Anticaries Efficacy of an Essential Oil Fluoride Mouthrinse Using an Intraoral Caries Test Model (Study No. 936-9213)**

Protocol Number:	**936-9213**
Research Report Number:	**936-0124**
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Sponsor:	**Oral Care** **Pfizer Consumer Healthcare R&D** **Pfizer Inc**
Study Initiation Date:	**October, 2000**
Study Completion Date:	**April, 2001**
Date of Report:	**January 16, 2002**

This study was performed in compliance with Good Clinical Practice (GCP) Guidelines.

TITLE: Determination of the Anticaries Efficacy of an Essential Oil Fluoride Mouthrinse Using an Intraoral Caries Test Model (Study No. 936-9213)

INVESTIGATORS: Dr. Domenick Zero, DDS, MS

STUDY CENTER: Oral Health Research Institute, Indiana University, Indianapolis.

STUDY PERIOD: First Enrollment: October, 2000

Last Completed: April, 2001

OBJECTIVES:

To determine the anticaries efficacy of an essential oil-containing mouthrinse with fluoride regimen using an intraoral caries test model for remineralization and fluoride uptake.

PURPOSE AND METHODS:

This observer-blind, randomized, controlled three-by-three crossover study used an intraoral caries test (ICT) model to assess mineral content change (Percent Surface Microhardness (%SMH) recovery), and fluoride uptake (as μg F/cm^2) in partially demineralized enamel specimen mounted on a partial denture. Three treatment regimens were: 1) a test EOF regimen (essential oil-containing mouthrinse with 0.02% sodium fluoride, rinsed with 20 ml solution for 30 seconds, BID), 2) a positive control regimen (sodium fluoride mouthrinse, 0.02% sodium fluoride, rinsed with 10 ml solution for 60 seconds, BID, compliant with FDA monograph) 3) a negative control EO regimen (essential oil-containing mouthrinse, rinsed with 20 ml solution for 30 seconds, BID).

Qualifying subjects started a two-to-three day "lead-in" period for their first randomly assigned treatment leg. During this period, each subject wore a mandibular partial denture and used the assigned mouth rinse and dosing regimen without supervision. At the conclusion of this "lead-in" period, two partially demineralized enamel specimens were mounted on the buccal flange area of each subject's partial denture. Subjects continued to use their assigned mouth rinse and dosing regimen for a two-week treatment period. Rinsing (twice daily for 2 weeks) was supervised in the morning on weekdays, and unsupervised in the evening and on weekends and holidays. At the end of the first treatment period, the specimens were removed for analysis, and the subjects started a washout period of at least 4 days prior to their second treatment phase. These procedures were repeated until each subject had completed all three treatments.

After each treatment period, the specimens were removed from the partial dentures. The samples were assessed for mineral content change using surface microhardness testing and then analyzed for fluoride uptake using a microdrill biopsy and fluoride electrode analysis.

NUMBER OF SUBJECTS:

153 subjects were enrolled; 152 subjects were randomized, of which 141 subjects had either complete or partial microhardness measurements and 125 subjects were considered evaluable.

MAIN CRITERIA FOR INCLUSION:

Volunteer subjects met the following criteria: aged 18 or older, good oral health, residence in a community with a fluoridated water supply (1 ppm F), not taking fluoride supplements, and use of a removable mandibular partial denture with sufficient room in the posterior buccal flange area to accommodate two enamel specimens. Subjects did not have any conditions requiring antibiotics during the study's treatment phase.

TEST PRODUCTS, DOSE, ADMINISTRATION AND DURATION OF TREATMENT:

Subjects were instructed to rinse twice daily with one of the following products and dosing regimens: 1) a test EOF regimen (essential oil-containing mouthrinse with 0.02% sodium fluoride, rinsed with 20 ml solution for 30 seconds), 2) a positive control regimen (sodium fluoride mouthrinse, 0.02% sodium fluoride, rinsed with 10 ml solution for 60 seconds, compliant with FDA monograph) 3) a negative control EO regimen (essential oil-containing mouthrinse, rinsed with 20 ml solution for 30 seconds). Subjects were instructed to rinse the whole mouth vigorously with the assigned mouthrinse (volume and time differing for each regimen). The duration of each treatment was 14 days.

CRITERIA FOR EVALUATION:

Efficacy variables: Efficacy was determined by evaluation of surface microhardness and enamel fluoride uptake in partially demineralized enamel specimens.

The primary efficacy variable was percent surface microhardness recovery (%SMH) after *in vivo* treatment. The secondary efficacy variable was Enamel fluoride uptake ($\mu g\ F/cm^2$) after *in vivo* treatment.

Safety: Adverse events were monitored during week-day visits for supervised rinsing or at the clinical examinations (Adverse events were also gathered from the washout periods at this time).

STATISTICAL METHODS:

The primary efficacy variable was percent surface microhardness recovery. The secondary efficacy variable was enamel fluoride uptake. For each of the primary and secondary efficacy variables, between-treatment differences were tested using a mixed model with

sequence, treatment, period, and carryover as fixed, and with subject as random.

The test fluoride mouthrinse (EOF) regimen was considered "at least as good as" the positive control (NaF rinse) regimen in promoting enamel remineralization if,

i) the mean % SMH recovery for the EOF test mouthrinse was statistically significantly greater than the mean % SMH recovery for the EO rinse negative control rinse, based on a two-sided 0.05-level test of the null hypothesis that the treatment means are equal versus the alternative hypothesis that the means are different, and

ii) for %SMH recovery, the lower limit of the one-sided 97.5% confidence interval for the difference between the EOF rinse test and NaF rinse positive control means (expressed as a percentage difference relative to positive control) was above –20% This procedure is a 0.025 level test of the null hypothesis that the mean for the test mouthrinse is at least 20% lower than the mean for the positive control, versus the alternative hypothesis that the mean for the test mouthrinse is not at least 20% lower than the mean for the positive control.

The study would be considered validated if the %SMH recovery for the positive control was both statistically significantly greater than the %SMH recovery for the negative control group, and the difference in mean %SMH recovery between the positive control and negative control was 10 %SMH units or greater (e.g., if the negative control rinse exhibited a 10% recovery in SMH, the positive control needed to exhibit a recovery in SMH of 20% or greater).

Enamel fluoride content was also evaluated by comparing the EOF test rinse with the EO rinse negative control.

SUMMARY/CONCLUSION:

Efficacy Results: EOF mouthrinse was effective in increasing both %SMH recovery and fluoride uptake by a) a statistically greater amount than the EO negative control, and b) "at least as good as" the NaF positive control. The study was considered validated since a) the mean %SMH recovery for positive control was statistically greater than that for the negative control and b) the difference of mean % SMH recovery between the positive control and negative control was 20 %SMH units (greater than the 10 %SMH units as agreed between the sponsor and FDA).

Safety Results: Adverse events were generally mild or moderate in nature. There were no deaths or other significant adverse events. One serious adverse event was reported for Subject #115 with chest pain but was judged unrelated to study drug. Four events were judged as either probably related to study drug (2 events; ulceration of the tongue and multiple ulcerations of the buccal mucosa), or possibly related to study drug (2 events; nausea and mouth soreness). Other events were judged as unlikely related to study drug. The

predominant adverse events reported by the subjects related to the digestive system and the rates were similar between the treatment groups. In general there were fewer events reported during the washout periods.

Conclusions:

The anticaries efficacy of the essential oil-containing mouthrinse with fluoride with its own dosing regimen was greater than the essential oil-containing mouthrinse without fluoride and was "at least as good as" the FDA monograph-compliant sodium fluoride mouthrinse with its own dosing regimen for promoting enamel remineralization and fluoride uptake.

Table
All Evaluable Subjects (n=125)

	% SMH			Fluoride Uptake (μg F/cm^2)		
	Negative Control	Test Product	Positive Control	Negative Control	Test Product	Positive Control
Adjusted mean	15.64	41.77	36.05	3.08	19.38	16.11
Difference vs. Negative Control		26.13	20.40		16.30	13.03
S.E. of Difference		1.83	1.85		0.91	0.92
p-value vs. Negative Control		<0.001	<0.001		<0.001	<0.001
Lower one-sided 97.5% confidence limit for Test vs. Positive Control, as % of Positive Control		5.9%			9.3%	